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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 09/589,870	Applicant(s) GOSHORN ET AL.	
	Examiner Stephen L. Rawlings, Ph.D.	Art Unit 1642	

-- The MAILING DATE of this communication app ars on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05 April 2001* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-66* is/are pending in the application.

4a) Of the above claim(s) *1-17, 40-64, and 66* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *18-39 and 65* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claims *1-66* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

15) ☒ Notice of References Cited (PTO-892)
16) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *5, 6, 7* .
18) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
19) ☐ Notice of Informal Patent Application (PTO-152)
20) ☐ Other: .

DETAILED ACTION

1. Claims 1-66 are pending in the application. Claims 1-17, 40-64, and 66 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention. Claims 18-39 and 65 are currently under prosecution.

Election/Restrictions

2. Restriction to one of the following inventions is required under 35 U.S.C. 121:

Group I. Claims 1-17 and 66, drawn to a vector construct and host cell comprising said vector construct, classified in class 435, subclass 320.1 and subclass 325.

Group II. Claims 18-39 and 65, drawn to a fusion protein, classified in class 530, subclass 391.1.

Group III. Claims 40-63, drawn to a method for targeting a tumor cell, classified in class 424, subclass 138.1.

Group IV. Claim 64, drawn to a method for constructing a tetravalent antibody, classified in class 435, subclass 69.7.

3. The inventions are distinct, each from the other because of the following reasons:

Inventions in Groups I and II are disclosed as biologically and chemically distinct, unrelated in structure and/or function, and/or made by and/or used in different methods and therefore, the claimed products are distinct.

Inventions in Groups III and IV are disclosed as materially different methods that differ at least in objectives, method steps, reagents and/or doses and/or schedules used, response variables, assays for end products and/or results, and criteria for success and therefore, the claimed methods are distinct.

Inventions II and III/IV are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially

different product or (2) the product as claimed can be used in a materially different process of using that product (MPEP § 806.05(h)). In the instant case the product fusion protein as claimed can be used in a materially different process of using that product, such as an affinity chromatography.

The inventions in groups I and IV are not at all related because the products of group I are not specifically used in any of the steps of the claimed methods in group IV.

4. Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification, restriction for examination purposes as indicated is proper.

5. During a telephone conversation with William T. Christiansen, Ph.D. on March 30, 2001 a provisional election was made with traverse to prosecute the invention of Group II, claims 18-39 and 65. Affirmation of this election must be made by applicant in replying to this Office action. Claims 1-17, 40-64, and 66 have been withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

6. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a petition under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Specification

7. The use of the trademark Primatized™ has been noted in this application. It should be capitalized wherever it appears and be accompanied by the generic terminology.

Although the use of trademarks is permissible in patent applications, the proprietary nature of the marks should be respected and every effort made to prevent their use in any manner that might adversely affect their validity as trademarks.

Claim Objections

8. Claim 32 is objected to because of the following informalities:

Claim 32 recites the limitation "the linker comprises at least four Gly_4Ser linkers". This recitation contains an amino acid sequence of more than four amino acid residues, namely GGGGS. Therefore, the claim contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR § 1.821(a)(1) and (a)(2). However, the claim is not written in a manner that complies with the requirements of 37 CFR § 1.821-1.825. Appropriate correction is required.

Claim Rejections - 35 USC § 112

9. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

10. Claims 18-39 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the single-chain antibody-streptavidin fusion proteins huNR-LU-10 scFvSA and B9E9 scFvSA, does not reasonably provide enablement for any other fusion protein. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The claims are drawn to a fusion protein comprising at least a first and a second polypeptide joined end to end, wherein the first polypeptide comprises at least 129

amino acids of streptavidin and wherein the second polypeptide differs from the first polypeptide by at least one amino acid residue. The claims are also drawn to said fusion protein wherein the second polypeptide is an antibody, a fragment of an antibody, or a single-chain antibody, wherein the variable heavy chain and the variable light chain of the single-chain antibody is separated by a linker, wherein said linker is composed of at least 4 gly_4-ser linkers. However, it is noted that the breadth of the claims encompasses a fusion protein comprising at least 129 amino acids of streptavidin and *any other polypeptide*. In terms of the single-chain antibody-streptavidin fusion protein, the claims encompass a fusion protein comprising a variable light chain and a variable heavy chain in any orientation and with any or no linker separating these two elements.

The specification teaches the production of two fusion proteins, both of which comprise a first polypeptide comprising amino acids 25 to 159 of streptavidin (SEQ ID NO: 2) and a second polypeptide comprising a single-chain Fv antibody derived from the recombinant humanized antibody huNR-LU-10 or the mouse monoclonal antibody B9E9 (Figures 1 and 2). The specification exemplifies the construction of the fusion proteins, huNR-LU-10 scFvSA and B9E9 scFvSA, respectively, consist of the 25-159 amino acid fragment of streptavidin and a single-chain antibody separated by a 5 amino acid linker (pages 29-36, Examples I-III). Furthermore, the single-chain antibodies of the exemplified fusion proteins consist of a variable light chain separated from a variable heavy chain by a linker composed of 3 gly_4-ser motifs (see, for example, Figure 11C). The specification also exemplifies the construction of a single-chain B9E9 antibody-streptavidin fusion protein in which the orientation of the variable light and heavy chains are reversed relative to the other construct, wherein the variable light and heavy chains are separated by a linker of 5 gly_4-ser motifs (page 32).

The specification teaches that the latter construct expressing the fusion protein consisting of a variable heavy chain joined by the linker composed of 5 gly_4-ser motifs to a variable light chain, each derived from the monoclonal antibody B9E9, and joined to the 25-159 amino acid fragment of streptavidin produces the greatest quantity of protein (page 37, Table 1). It is noted that some of the constructs, particularly those in which

the orientation of the variable heavy and light chains are reversed relative to the high yielding construct, produce very little fusion protein (page 37, Table 1).

In Example V (pages 38-39) the specification teaches that the expression level of some, but not all fusion proteins comprising a single-chain antibody and streptavidin increases in the presence of the *E. coli FkpA* gene product; however it is noted that the expression of two fusion proteins actually decreased in the presence of the *E. coli FkpA* gene product (page 39, Table 2). In Example VI (pages 40-41) the specification teaches a method for purifying the exemplified fusion proteins. In Example VII (pages 41-45), the specification teaches that the single-chain antibody-streptavidin fusion proteins retain some of the binding characteristics of the parental antibodies from which the fusion proteins are derived and retain biotin-binding activity. Finally, in Examples VIII-X (pages 45-48) the specification teaches a method for determining the biodistribution and measuring blood clearance of the single-chain antibody-streptavidin fusion proteins after administration to mice.

The teachings of the specification cannot be extrapolated to the enablement of the invention commensurate in scope with the claims because in view of the unpredictability in the art, there is insufficient guidance and exemplification that is commensurate in scope with the claims. Therefore, one skilled in the art cannot make and use the invention commensurate in scope with the claims with a reasonable expectation of success without first performing extensive and undue experimentation. The reason is set forth below:

The claims are drawn to a fusion protein comprising streptavidin and any second polypeptide that differs from streptavidin by at least one amino acid residue. However, the specification does not teach methods for producing and using a fusion protein comprising streptavidin and any second polypeptide, except in the embodiment wherein the second polypeptide is a single-chain antibody. For example, the specification does not teach how to make and use a fusion protein comprising streptavidin and a variant form of streptavidin that differs in least one amino acid residue. While the teachings of the specification are deemed insufficient because one skilled in the art cannot immediately appreciate the utility of a fusion protein comprising streptavidin and *any*

polypeptide, it is apparent that not every polypeptide will retain its functionality when simply fused to streptavidin. A number of the claimed fusion proteins would have to be engineered in order to produce a bifunctional protein with the functional characteristics of both polypeptides of which the fusion protein is composed, a manufacturing process that requires considerable time and expense. Because of the unpredictability in the art of protein engineering, one skilled in the art would not reasonably expect to successfully make the invention commensurate in scope with the claims having only the guidance and examples provided in the specification and would be forced to first perform undue experimentation. With regard to chimeric fusion proteins, Chilkoti, et al (*Bio/Technology* **13**: 1198-1204, 1995) teach potential limitations and problems associated with engineering bifunctional fusion proteins containing streptavidin. Chilkoti, et disclose that "the degeneracy of the four high-affinity subunits in the streptavidin tetramer can be detrimental from several key perspectives. With steptavidin fusion proteins, for example, the self-assembly of the tetramer dictates that the final product will contain four fusion domains. The functionality of the fusion tetramer may not require four such domains, and the presence of extra fusion domains may increase undesirable non-specific interactions or sterically hinder the biotin-binding site. The homotetrameric structure of streptavidin also precludes subunit-specific cross-linking of therapeutics/imaging agents/antibodies, since the reactive side-chains are equeally distributed across the individual subunits in the tetramer. Furthermore, the extremely high affinity of the streptavidin subunits for biotin, while crucial to many applications, can be detrimental in applications where reversible immobilization of streptavidin or biotinylated targets is ultimately desirable" (page 1198, columns 1-2). Chilkoti, et al also disclose that "in drug-delivery applications where streptavidin-biotin system forms the targeting and/or delivery component, the exceptionally slow biotin dissociation kinetics limits the potential applications utilizing diffusion of the biotinylated imaging agent or drug to the therapeutic target, and may also result in unfavorably slow *in vivo* clearance of biotinylated imaging agents" (page 1198, column 2). In view of the teachings of Chilkoti, et al it is clear that one skilled in the art cannot predict whether the claimed invention can be made and used successfully. While the applications of the claimed

invention are variable, the limitations that Chilkoti, et al disclose suggest that the utility of the invention can only be determined empirically. For example, without experimentation, it cannot be determined whether a fusion protein comprising streptavidin will retain the ability to form tetramers and to bind biotin. It is possible that the second polypeptide of which the fusion protein is composed will sterically hinder multimerization of the streptavidin moieties and also block binding of biotin. Accordingly, in the absence of exemplification commensurate in scope with the claims, one skilled in the art would be forced to perform undue experimentation in order to make and/or use the invention with a reasonable expectation of success.

The claims are drawn to a fusion protein comprising streptavidin and any fragment of an antibody. However, the specification does not teach a method for producing and using a fusion protein comprising streptavidin and any fragment of an antibody. In the absence of exemplification, one skilled in the art cannot immediately appreciate the utility of a fusion protein comprising streptavidin and *any* fragment of an antibody, particularly if said fragment does not have antigen-binding activity.

The claims are also drawn to a fusion protein comprising streptavidin and any single-chain antibody in which the variable light chain and the variable heavy chain are or are not separated by a linker. However, one skilled in the art would not expect a fusion protein comprising a single-chain antibody in which the variable light and heavy chains are *not* separated by a spacer to function; yet the claims encompass such a fusion protein. It is well known in the art that a flexible, hinge-like linker separating the variable light and heavy chains of a single-chain antibody is required for proper folding and function. Also, one skilled in the art might not expect a fusion protein comprising a single-chain antibody in which the variable light and heavy chains are separated by a linker of 100 amino acids or more to function; yet the claims encompass such a fusion protein. In actuality, one skilled in the art cannot predict whether a given fusion protein comprising a single-chain antibody in which the variable light and heavy chains are separated by a linker will function, because the length and composition of the linker has been determined to be critical. Some linkers have become fairly conventional in the art, such as the 15 amino acid linker composed of 3 gly_4-ser motifs, the use of which is

exemplified in the instant application. See, for example, Desplanqc, et *al (Protein Engineering* **7**: 1027-1033, 1994; Form PTO-1449, Paper No. 6, page 3). Nevertheless, the claims are clearly drawn to fusion proteins comprising a single-chain antibody in which the variable light and heavy chains are separated by *any* linker. However, Tang, et al (*Journal of Biological Chemistry* **271**: 15682-15686, 1996) teach that "successful construction of an scFv depends upon the choice of a linker that neither interferes with the folding and association of the V_H and V_L domains nor reduces the stability and recognition properties of the Fv molecule" (page 15682, column 2). It is evident from the teachings of Tang, et al that there are many linkers that cannot be used successfully in the construction of a scFv. With regard to linkers, Tang, et al conclude, "subtle differences in sequence dramatically influence the production, stability, and recognition properties of the scFv" (abstract). Consistently, Desplanqc, et al (cited supra) teach that "very considerable differences in binding activity between scFvs with different linker lengths and with different relative orientations of V_L and V_H" occur (page 1030, column 2). Desplanqc, et al compared the binding activities of single-chain antibodies comprising a variable light chain and a variable heavy chain in different orientations relative to the other single-chain antibodies. Desplanqc, et al teach that for the V_H-linker-V_L organization, "only the variant with a 30 amino acid linker showed good binding activity" (abstract), suggesting that the amino- or carboxyl-termini of single-chain antibodies make different structural contributions to antigen binding. This feature will, of course, vary depending upon the antibody and therefore one skilled in the art cannot predict whether the claimed fusion proteins can be used without first performing undue experimentation.

It is also evident that the composition and the length of the linker determines whether the single-chain antibody can be produced in sufficient quantity and purified to be useful. Shultz, et al (*Cancer Research* **60**: 6663-6669, 2000) teach that "most scFVSA fusion proteins are poorly expressed or insoluble in the periplasmic space" (page 6668, column 1). With regard to the claimed fusion protein composed of streptavidin and a single-chain antibody derived from the monoclonal antibody B9E9, Shultz, et al teach that "efforts to produce high levels of a V_L-V_H B9E9 scFvSA with a

15-mer Gly4Ser linker were unsuccessful" (page 6668, column 1). In light of the teachings of Schultz, et al, it is clear that this fusion protein cannot be produced in sufficient quantity to be useful; yet it is noted that the claims encompass this particular fusion protein. As noted above, the teachings of Desplanqc, et al are an indication that the length and composition of the linker separating the variable heavy and light chains of a single-chain antibody is a critical determinant of the binding activity of the antibody. Desplanqc, et al teach that "the efficiency of purification correlated with antigen binding ability for each scFv" (page 1029, column 1). Therefore, it is clear that purification of the claimed fusion proteins may preclude their usefulness if the fusion protein does not retain a high enough affinity for the antigen. Desplanqc, et al also teach that the length and composition of the linker influences whether the single-chain antibodies multimerize and "multimerization may lead to precipitation" (page 1030, column 1), which can obviously limit the usefulness of a single-chain antibody. Desplanqc, et al disclose that "scFvs with linkers of 30 amino acids (or perhaps even longer) may give the best chance of minimizing the tendency to form multimers while retaining antigen binding activity" (page 1033, column 1). While the construction of a scFv with a 30 amino acid linker may overcome some of the problems with production, Desplanqc, et al also teach that "long linkers may, however, also confer upon scFvs the potential disadvantages in patients of enhanced immunogenicity and susceptibility to protease cleavage" (page 1033, column 1). Therefore, once a single-chain antibody-streptavidin fusion protein is produced in sufficient quantity, one skilled in the art cannot predict whether the single-chain antibody-streptavidin fusion protein can be used effectively to treat patients. Thus, it is clear that undue experimentation would be required of the skilled artisan to use the invention in a clinical setting.

Additionally, it is noted that the claims are drawn to a fusion protein comprising streptavidin and a single-chain antibody comprising a variable light chain and a variable heavy chain in any orientation. However, it is apparent from Table 1 (page 37) that the fusion proteins in which the single-chain antibody consists of a variable light chain joined to a variable heavy chain, which is joined to streptavidin (i.e., V_H-V_L-SA), are not produced in sufficient quantities to be useful. Desplanqc, et al (cited supra) also teach

that orientation of the variable light and variable heavy domains of the single-chain antibody can dramatically influence its antigen-binding affinity. Again, one skilled in the art cannot predict which fusion proteins comprising a variable light chain and a variable heavy chain in any orientation will be capable of being produced in sufficient quantity to be useful or whether such fusion proteins will be stable and retain the recognition properties of the parental antibody.

Therefore, in view of the unpredictability in the art of protein and antibody engineering, in the absence of sufficient guidance and exemplification commensurate in scope with the claims, one skill in the art cannot make and use the invention with a reasonable expectation of success without undue experimentation. For this reason, the specification provides reasonable enablement only for claims drawn specifically to the single-chain antibody-streptavidin fusion proteins huNR-LU-10 scFvSA and B9E9 scFvSA comprising a 15 and 25 amino acid gly_4-ser linker, respectively, the production and use of which are exemplified in Examples I-X.

11. Claim 65 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claim is drawn to a pharmaceutical composition comprising a fusion protein according to any one of claims 18-39.

The specification teaches the biodistribution of the single-chain antibody-streptavidin fusion protein huNR-LU-10 scFvSA after administration to nude mice bearing human colon cancer xenografts in Example VIII (pages 45-47). The single-chain antibody huNR-LU-10, from which the fusion protein is derived, binds specifically to an antigen that is displayed at the surface of many cancer cells, including some colon cancer cells. The specification teaches that the fusion protein can be detected by administering to the mice ^{111}In-DOTA-biotin after the fusion protein has been administered, indicating that the antibody-streptavidin fusion protein maintains the capacity to bind biotin. Because radiolabeled biotin binds the fusion protein, the

measured level of radioactivity in blood or other tissues dissected from the mice is indicative of the concentration of the fusion protein in that tissue. According to the specification, the highest concentration of fusion protein was found in the xenograft tissue (i.e., human colon cancer). Very low or undetectable levels of the fusion protein were found in non-xenograft tissues (i.e., mouse tissues). See Figure 16. These data indicate that the antibody-streptavidin fusion protein maintains the binding specificity of the parental antibody from which the fusion protein is derived.

The specification also exemplifies the biodistribution of another single-chain antibody-streptavidin fusion protein, namely B9E9 scFvSA, in Example X (page 48). The specification teaches that the single-chain antibody-streptavidin fusion protein is administered to nude mice bearing human cancer xenografts; however, in this case, the Ramos cancer cell line was used to establish tumors in the mice. The single-chain antibody B9E9, from which the fusion protein is derived, binds specifically to CD20, an antigen that is displayed at the surface of Ramos cells. Here again, the specification teaches that ^{111}In-DOTA-biotin is concentrated at the site of the human xenograft tumor and not in non-xenograft tissues of the mice injected with B9E9 scFvSA (Figure 18), indicating that the antibody-streptavidin fusion protein maintains the binding specificity of the parental antibody from which the fusion protein is derived and maintains the capacity to bind biotin.

The teachings of the specification cannot be extrapolated to the enablement of the claims because the specification does not teach how one skilled in the art can use a pharmaceutical composition comprising a fusion protein according to any one of claims 18-39. Clearly, not all fusion proteins encompassed by the claims can be formulated as a pharmaceutical composition and used to treat or diagnose a medical condition in a subject, particularly a human subject. For example, the claims encompass a fusion protein comprising a first and a second polypeptide joined end to end, wherein the first polypeptide comprises at least 129 amino acids of streptavidin and the second polypeptide comprises at least 129 amino acids of streptavidin wherein one amino acid residue has been substituted for another amino acid residue so that the second polypeptide differs from the first polypeptide. However, the specification does not teach

how such a fusion protein, when formulated as pharmaceutical composition, can be used to treat or diagnose a medical condition in a subject. Moreover, one of skill in the art would not expect that a pharmaceutical composition comprising such a fusion protein could be used to treat or diagnose a medical condition. The claims also encompass a fusion protein comprising a first and a second polypeptide joined end to end, wherein the first polypeptide comprises at least 129 amino acids of streptavidin and the second polypeptide comprises a ligand, which binds a cell surface receptor. However, the specification does not exemplify the use of a pharmaceutical composition comprising a fusion protein comprising a first and a second polypeptide joined end to end, wherein the first polypeptide comprises at least 129 amino acids of streptavidin and the second polypeptide comprises a ligand, which binds a cell surface receptor. Clearly, one skilled in the art cannot predict whether a fusion protein comprising a first and a second polypeptide joined end to end, wherein the first polypeptide comprises at least 129 amino acids of streptavidin and the second polypeptide comprises a ligand, which binds a cell surface receptor, can be used effectively to treat or diagnose a medical condition in a subject, particularly a human subject. For that matter, one skilled in the art cannot predict that a streptavidin-ligand fusion protein will retain the capacity of the parent ligand to bind specifically to the receptor for that ligand, a property of the fusion protein which can only be determined empirically. Nevertheless, there are, of course, many more reasons why one skilled in the art would expect to find many non-working embodiments of the claimed invention. For example, not all ligands will bind a receptor that is differentially expressed by a tumor cell, such that the pharmaceutical composition will target biotin-conjugated chemotherapeutic agents specifically to tumor cells and not to other non-tumor cells. While it is perhaps acceptable that the claims are drawn to a certain number of non-working embodiments, there are clearly hundreds and hundreds of embodiments that would not be expected to work, but which are encompassed by the claims. There are also hundreds and hundreds of embodiments that might reasonably be expected to work, because there are no obvious reasons to suspect that such embodiments might not work. However, because of the unpredictability in the art, one skilled in the art cannot predict whether any one of these

reasonable embodiments can be used effectively to treat or diagnose a medical condition in a subject, particularly a human subject. Furthermore, it is noted that the specification does not exemplify the use of even one of species of pharmaceutical compositions in the claimed genus. Moreover, the specification does not provide guidance as to which medical conditions might be treated or diagnosed with which of the claimed pharmaceutical compositions.

On the other hand, there is an inference that the huNR-LU-10 scFVSA and B9E9 scFvSA can be used to diagnose or treat a patient with cancer, since both antibodies from which the fusion proteins are derived specifically bind an antigen, the expression of which is associated with cancer. However, the specification does not actually exemplify the formulation of these single-chain antibody-streptavidin fusion proteins as pharmaceutical compositions. Moreover, the specification does not exemplify the use of the single-chain antibody-streptavidin fusion proteins to diagnose or treat a medical condition, including cancer. Again, the examples in the specification serve only to illustrate that the fusion proteins retain the capacity to bind biotin and retain the binding specificity of the parental antibody from which they are derived. Because the art of cancer therapy is highly unpredictable, one skilled in the art cannot predict which, if any of the claimed multitudes of pharmaceutical compositions, including those that comprise huNR-LU-10 scFVSA and B9E9 scFvSA, will in fact work without first performing extensive and undue experimentation.

Factors to be considered in determining whether undue experimentation is required, are summarized in *Ex parte Forman*, 230 USPQ 546 (BPAI 1986). They include the nature of the invention, the state of the prior art, the relative skill of those in the art, the amount of direction or guidance disclosed in the specification, the presence or absence of working examples, the predictability or unpredictability of the art, the breadth of the claims, and the quantity of experimentation which would be required in order to practice the invention as claimed.

It is well known that the art of anticancer drug discovery for cancer therapy is highly unpredictable. For example, Gura (*Science* **278**: 1041-1042, 1997) teaches that researchers face the problem of sifting through potential anticancer agents to find ones

promising enough to make human clinical trials worthwhile (abstract). Gura teaches that since formal screening began in 1955, many thousands of drugs have shown activity in either cell or animal models, but that only 39 have actually been shown to be useful for chemotherapy (page 1041, first and second paragraphs).

Furthermore, the refractory nature of cancer to drugs is well known in the art. Jain (*Scientific American* **271**: 58-65, 1994) teaches that most tumors resist full penetration by anticancer agents (page 58, column 1) and that scientists need to put expanded effort into uncovering the reasons why therapeutic agents that show encouraging promise in the laboratory often turn out to be ineffective in the treatment of common solid tumors (page 65, column 3). Curti (*Critical Reviews in Oncology/Hematology* **14**: 29-39, 1993) teaches that solid tumors resist destruction by chemotherapy agents and that although strategies to overcome defense mechanisms of neoplastic cells have been developed and tested in a number of patients, success has been limited. Curti also teaches that it is certainly possible that cancer cells possess many as yet undefined additional molecular mechanisms to defeat chemotherapy treatment strategies and, if this is true, designing effective chemotherapeutic regimens for solid tumors may prove a daunting task (paragraph bridging pages 29-30). Curti concludes that knowledge about the physical barriers to drug delivery in tumors is a work in progress (page 36, column 2). Thus, it is clear that based on the state of the art, in the absence of experimental evidence, no one skilled in the art would accept the assertion that a pharmaceutical composition comprising any one of the multitudes of claimed fusion protein could be used effectively to treat or diagnose a medical condition or disease in a subject, based only upon the demonstrated biodistribution of two species of the claimed genus of fusion proteins.

In addition, anti-tumor agents must accomplish several tasks to be effective. They must be delivered into the circulation that supplies the tumor and interact at the proper site, and they must do so at a sufficient concentration and for a sufficient period of time so as to be effective. Also, the targeted cells must not have an alternate means of survival despite action at the proper site for the drug. In addition, variables such as biological stability, half-life, and clearance from the blood are important parameters in

achieving successful therapy. The composition may be inactivated *in vivo* before producing a sufficient effect, for example, by degradation, immunological activation, or due to an inherently short half-life. The composition may not otherwise reach the target because of its inability to penetrate tissues or cells where its activity is to be exerted. Alternatively, the composition may be absorbed by fluids, cells and tissues where the formulation has no effect and circulation into the target area may be insufficient to carry the composition and to permit a large enough local concentration to be established. The demonstration that the single-chain antibody-streptavidin fusion proteins concentrate at the sites of xenograft tumors in mice is insufficient evidence that the compositions comprising these fusion proteins can be used to effectively diagnose or treat cancer in a subject. The experiments performed in xenograft mouse models are artificial and it is well known in the art that the results of such experiments cannot be extrapolated to accurately predict whether a composition can also be used effectively in humans to diagnose or treat a disease such as cancer. Furthermore, there are well known limitations in the art of antibody-targeted diagnostic and therapeutic treatments and it cannot be predicted whether the claimed invention can be used to effectively overcome these limitations. Therefore, the efficacy of using the claimed invention can only be determined empirically.

In regard to antibody-targeted therapy, *per se*, Vitetta, et al (*Cancer Research* **54**: 5301-5309, 1994) teach that "despite [...] intellectual appeal, the general therapeutic efficacy of tumor-reactive MAbs [monoclonal antibodies] has been disappointing. In particular the results of clinical studies in patients with solid tumors showed little efficacy, except in the setting of minimal disease" (citations omitted) (page 5301, column 1). Vitetta, et al continue, teaching that there are a number of significant limitations in their use as first-line therapy for solid tumors page 5305, (columns 1-2):

> Only 0.001 to 0.1% of injected MAb [monoclonal antibody] will localize to each [gram] of tumor mass. Moreover, MAbs, even at high serum concentrations, cannot gain access to all the cells in solid epithelial tumor. The reasons for this are poor and heterogeneous blood supply, the blood-tumor barrier, and the selective binding of the MAb by the tumor cells closest to the blood supply. In addition, MAbs by themselves probably cannot kill the 10^{10}-10^{12} malignant cells that may be necessary to cure a patient with a disseminated tumor (citations omitted) (page 5305, columns 1-2).

The use of a pharmaceutical composition comprising any one of the multitudes of claimed fusion proteins that comprise a single-chain antibody will, of course, be subject to the same limitations that any antibody-targeted therapy will have. Therefore, in the absence of exemplification demonstrating that any one of the multitudes of claimed fusion proteins that comprise a single-chain antibody can be used effectively to treat or diagnose a medical condition in a subject, one skilled in the art cannot use such an invention with a reasonable expectation of success, in light of the teachings of Vitetta, et al, without first performing extensive and undue experimentation, because of the art's unpredictability.

Consistently, US 6,156,321 A teaches that "it is much more difficult for most chemotherapeutic agents to reach all of the cells of a solid tumor mass than it is the soft tumors and blood-based tumors, and therefore much more difficult to achieve a total cell kill" (column 1, lines 53-57). Then, there is another problem that frequently arises in treatment that would limit the effectiveness of a therapy, making it difficult, if not impossible to achieve a "total cell kill". It is well known in the art that tumor cells often undergo a selective process that ultimately leads to the formation of a mass of cells that is no longer sensitive to the chemotherapeutic agent first used to treat the primary tumor.

Furthermore, the strategic approach to treating cancer using antibody therapy, which is asserted in the instant application, is analogous to active specific immunotherapy (e.g., vaccination against tumor-associated antigens), at least to the extent that the latter theoretically induces a humoral immune response (i.e., the production of tumor-specific antibody). Antibody therapy can be defined as passive immunization, cancer vaccine therapy as active immunization. Because the efficacy of both approaches depends upon the effectiveness of tumor antigen-specific antibodies to ameliorate or inhibit tumors, both also share the same or corresponding limitations. Bodey, et al (*Anticancer Research* **20**: 2665-2676, 2000) teach that "while cancer vaccine trials have yielded tantalizing results, active immunotherapy has not yet become an established modality of anticancer therapy" (page 2665, column 2) and "the

use of active specific immunotherapy (ASI) for cancer (cancer 'vaccines') is still in its scientific infancy despite several decades of clinical and basic research" (page 2668, column 2). In the abstract Bodey, et al disclose:

> Animal models, albeit highly artificial, have yielded promising results. Clinical trials in humans, however, have been somewhat disappointing. Although general immune activation directed against the target antigens contained with a cancer vaccine has been documented in most cases, reduction in tumor load has not been frequently observed, and tumor progression and metastasis usually ensue, possibly following a slightly extended period of remission. The failure of cancer vaccines to fulfill their promise is due to the very relationship between host and tumor: through a natural selection process the host leads to the selective enrichment of clones of highly aggressive neoplastically transformed cells, which apparently are so dedifferentiated that they no longer express cancer cell specific molecules. Specific activation of the immune system in such cases only leads to lysis of the remaining cells expressing the particular TAAs [tumor associated antigens] in the context of the particular human leukocyte antigen (HLA) subclass and the necessary costimulatory molecules. The most dangerous clones of tumor cells however lack these features and thus the cancer vaccine is of little use.

Clearly, if a tumor does not express the antigen that is specifically bound by the antibody component of the fusion protein the use of a pharmaceutical composition comprising a fusion protein derived from that antibody will not be effective. As Bodey, et al teach, use of such a pharmaceutical composition may only serve to select against tumor cells that express the antigen, while promoting the growth of tumor cells that do not express the antigen. Thus, while the efficacy of a pharmaceutical composition that comprises one of the claimed fusion proteins that comprises an antibody cannot be predicted by one skilled in the art without undue experimentation, clearly one skilled in the art will have reason to doubt that the pharmaceutical composition can be used effectively, in light of the teachings of Bodey, et al.

The use of antibody-streptavidin fusion proteins that target radiolabeled biotin or chemotherapeutic agents conjugated to biotin to diseased cells for treatment of a medical condition in a subject is analogous to the use of monoclonal antibodies conjugated to chemotherapeutic agents, such as radioisotopes. With regard to treatment strategies that utilize such pharmaceutical compositions to treat patients diagnosed with a tumor, DeNardo, et al (*Clinical Cancer Research* **5**: 3219s-3223s, 1999) teach "single-agent radioimmunotherapy (RIT) has proven efficacy as a treatment for hematological malignancies, particularly non-Hodgkin's lymphoma. Although

promising, RIT has been less effective for solid tumors, in part because they are less radiosensitive" (abstract). Thus, it is clear that there is much contention in the art that antibody-based therapies are presumed ineffective until proven otherwise, and just as clear, there is the lack of predictability in the art of cancer diagnostics and therapy.

Finally, the teachings of the specification cannot be extrapolated to the enablement of full scope of the invention, because clearly the claimed method cannot be used to effectively treat or diagnose a medical condition in a subject, if the diseased cells that are to be treated or detected do not express the antigen to which the single-chain antibody binds, or if the antigen is expressed at too low a level. For example, Railo, et al (*European Journal of Cancer* **30A**: 307-311, 1994) determined that 39% of biopsied specimens isolated from patients diagnosed with breast cancer expressed the insulin-like growth factor-I receptor (IGF-IR) (abstract); accordingly, it would be expected that 61% of the patients studied by Railo, et al would not respond to anti-IGF-IR single-chain antibody-streptavidin fusion protein therapy. Because the tumors in these patients did not express IGF-IR, these tumors would not bind the antibody portion of the fusion protein and therefore would not be targeted by a biotin-conjugated chemotherapeutic agent. The specification, however, is silent with regard to this issue and does not provide guidance for patient selection based upon any reasonable criteria. On the other hand, because IGF-IR is expressed by normal, non-cancerous cells in many tissues, there is justifiable concern in the art that administering an antibody-streptavidin fusion protein that targets a chemotherapeutic agent to cells that express IGF-IR may lead to non-selective cytotoxic effects, killing normal cells as well as tumor cells and possibly causing an "autoimmune-like" disease, such as diabetes.

Therefore, the invention is not enabled to the full breadth of the claims by the teachings of the specification because one of skill in the art cannot predict whether claimed pharmaceutical compositions can be used successfully to effectively treat a medical condition or disease in a human patient. The specification does not actually teach the application of the claimed pharmaceutical composition in which the subject treated with the composition is a human. Because there are no working examples (or for that matter, prophetic examples) which demonstrate (or anticipate) that the claimed

method can be used to effectively treat a medical condition in a patient, in view of the high level of unpredictability in the art of cancer therapy, one skilled in the art cannot predict the efficacy of the invention or, in other words, whether the invention can be used clinically. Therefore, one skilled in the art cannot practice the invention with a reasonable expectation of success without being forced to perform undue experimentation. The specification has not exemplified the diagnosis of a tumor in mouse or human subjects. The specification has not demonstrated that the fusion proteins can be used to effectively treat subjects diagnosed with tumors. While the specification teaches that the biodistribution of two single-chain antibody-streptavidin fusion proteins after administration to mice bearing xenograft tumors, it is clear that one skilled in the art would not expect to be able to inject live tumor cells into humans in order to practice the invention as claimed. One skilled in the art will not accept the assertion that pharmaceutical compositions comprising these single-chain antibody-streptavidin fusion proteins can be used to effectively diagnose and treat a tumor in a subject, based only upon the disclosure that the fusion proteins maintain binding capacity and specificity. Actually, there is no evidence of record that there is any clinical significance to the finding that the single-chain antibody-streptavidin fusion protein is distributed specifically to the xenograft tumor in mice. Also, it is noted that an effective dose for a mouse will be vastly different from an effective dose for a human or any other large mammal. Yet, the specification is silent with regard to this issue, making no reference to an appropriate dose and/or schedule for treatment of a human subject.

In summary, there is no factual evidence of record that indicates that any one of the multitude of claimed pharmaceutical compositions can be used to effectively prevent, treat, or diagnose a primary tumor or metastasis in a human or any other mammal. As such, the specification provides insufficient guidance to enable one skilled in the art to practice the invention. With regard to cancer therapy, undue experimentation would be required to determine how much or how often antibody must be delivered to a human or any other subject to effect a suitable level of tumor growth inhibition, and whether or not such dosages are tolerable to patients, without causing non-specific toxicity. With regard to other medical conditions, the specification does not

teach which of the claimed fusion proteins can be used to effectively diagnose or treat other diseases. In the absence of exemplification commensurate in scope with the claims, which teaches that the claimed invention can be used effectively to treat or diagnose a medical condition in any subject, particularly a human subject, because of the lack of predictability in the art, one skilled in the art cannot practice the invention with a reasonable expectation of success without undue experimentation.

12. Claims 18-22, 38, 39, and 65 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 18-22, 38, and 39 are drawn to a fusion protein comprising a first and a second polypeptide, wherein the first polypeptide comprises at least 129 amino acids of streptavidin and the second polypeptide differs from the first polypeptide by at least one amino acid residue. Claim 65 is drawn to a pharmaceutical composition comprising the fusion protein of claims 18-22, 39, or 39.

As broadly written, the claims are drawn to an enormous genus of fusion proteins or pharmaceutical compositions comprising these fusion proteins. However, in this case, the written description only sets forth methods for producing two species of the fusion proteins, both of which are single-chain antibody-streptavidin fusion proteins. The specification is devoid of exemplification of the production and use of any other species of fusion protein, which does not comprise an antigen-binding fragment of an antibody. Moreover, the specification is devoid of exemplification of the use of the claimed genus of fusion proteins to diagnose, prevent, or treat maladies in any subject.

The disclosure is deemed insufficient because it cannot be predicted by one of skill in the art which, if any of the species contained within the enormity of the claimed genus of fusion proteins and pharmaceutical compositions can be used effectively. One skilled in the art also cannot predict which of the claimed fusion proteins, which do not comprise an antigen-binding fragment of an antibody, can be made and used. Accordingly, the specification is devoid of teachings that would enable one of skill in the

art to use the claimed invention; thus, it would seem that Applicant did not actually possess an agent at the time of filing which could be used to practice the invention as claimed.

Vas-Cath Inc. V. Mahurkar, 19 USPQ2d 1111, clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed*" (page 1117). The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed" (page 1116).

Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 USC 112 is severable from its enablement provision (page 115).

Moreover, although drawn to the nucleic acid art, the findings of *Fiers v. Revel*, 25 USPQ 2d 1601 at 1606 (CAFC 1993) and *Amgen Inc. V. Chugai Pharmaceutical Co. Lts.*, 18 USPQ2d 1016 are clearly relevant to the instant invention. In *Fiers v. Revel* and *Amgen Inc. V. Chugai Pharmaceutical Co. Lts.* the court found that adequate written description requires more than a mere statement that it (a nucleic acid) is part of the invention. The nucleic acid itself is required; or in the instant case, the fusion protein or a pharmaceutical composition comprising said fusion protein is required.

Furthermore, although again drawn to the nucleic acid art, in *The Regents of the University of California v. Eli Lilly* (43 USPQ2d 1398-1412), the court held that a generic statement which defines a genus of nucleic acids by only their functional activity does not provide an adequate written description of the genus. The court indicated that while Applicants are not required to disclose every species encompassed by a genus, the description of a genus is achieved by the recitation of a representative number of DNA molecules, usually defined by a nucleotide sequence, falling within the scope of the claimed genus. At section B(1), the court states that "An adequate written description of a DNA...'requires a precise definition, such as by structure, formula, chemical name, or physical properties', not a mere wish or plan for obtaining the claimed chemical invention". Accordingly, there is an inference that an adequate description of a fusion protein or a pharmaceutical composition comprising said fusion protein also requires a

very precise definition, including an indication of its precise chemical and the quantity of said pharmaceutical composition that would be therapeutically effective. There is also an inference that the mere description of a genus of generically applicable agents, limited only by a disclosed generic statement relating the functional activities of the species, does not provide such an adequate written description of the genus. In this case, however, the written description merely sets forth that the claimed genus of fusion proteins has a pharmaceutical use. Of course, an adequate written description requires a disclosure of what malady or condition can be treated or diagnosed using the claimed invention. In the absence of such a disclosure, clearly one skilled in the art would not reasonably conclude that Applicant was in possession of the invention at the time the application was filed.

In the absence of sufficient written description, the Applicant merely appears to extend an invitation to those skilled in the art to discover which, if any members of the genus of fusion proteins can be made and used. Because the efficacy and applicability of the genus of fusion proteins is highly variant and because one of skill in the art would not have a reasonable expectation of success in practicing the invention as claimed without first testing the production and use of a fusion protein, the disclosure of two species of putatively effective fusion proteins that could be tested for utility in diagnosing or treating cancer is insufficient to support the generic claims as provided by the Interim Written Description Guidelines published in the June 15, 1998 Federal Register at Volume 63, Number 114, pages 32639-32645. Therefore, only the fusion proteins, which comprise an antigen-binding fragment of an antibody or a single-chain antibody, namely huNR-LU-10 scFvSA and B9E9 scFvSA, meet the written description requirement of 35 USC § 112, first paragraph.

13. The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

14. Claims 18-39 and 65 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 18-39 and 65 are indefinite because claims 18, 24, 38, and 39 recite the phrase "streptavidin, Figure 4". Figure 4 shows the complete amino acid sequence of polypeptide encoded by the gene together with the polynucleotide sequence of the gene. It is not immediately apparent in the figure to which sequence the claim refers and the numbering system used is confusing. Therefore, one of ordinary skill in the art is not reasonably apprised of the metes and bounds of the invention. Moreover, as the figure now appears, the Draftsman has not approved Figure 4. However, it is noted that SEQ ID NO: 2 sets forth just the amino acid sequence of streptavidin. Therefore, amending claim 18 to recite, for example, the phrase "at least 129 amino acids of streptavidin having the amino acid sequence set forth in SEQ ID NO: 2" can obviate this rejection.

Claims 18-39 and 65 are indefinite because claim 18 recites the phrase "a first and a second polypeptide joined end to end". The use of the phrase renders the claim indefinite because it is unclear whether the terms first polypeptide and second polypeptide are meant to indicate the amino-terminal polypeptide and the carboxyl-terminal peptide, respectively, or are merely meant to distinguish one from the other. The fact that the first and second polypeptides are joined end to end is not limiting with regard to the orientation of the polypeptides relative to one another within the fusion protein. Accordingly, one of ordinary skill in the art is not reasonably apprised of the metes and bounds of the invention. Amending claim 18 to recite, for example, the phrase "a first amino-terminal and a second carboxyl-terminal polypeptide joined end to end" can obviate this rejection.

Claims 18-39 and 65 are also vague and indefinite because claim 18 recites the phrase "functional variant". The use of the phrase renders the claim vague and indefinite because it cannot be ascertained what constitutes a functional variant of streptavidin. First of all, it is unclear to what function the claim refers (e.g., antigenicity, biotin binding, ability to form tetramers, etc.). Secondly, a functional variant of

streptavidin may be considered any a variant of streptavidin that ranges in the full extent to which a particular function is retained (i.e., from entirely non-functional to entirely functional). Therefore, one of ordinary skill in the art is not reasonably apprised of the metes and bounds of the invention. Amending the claim to recite a specific function and a specific value indicative of the degree to which said function is required to be retained may obviate this rejection. However, Applicant is cautioned against the introduction of new matter into the claims by amendment.

Claim 21, 22, and 65 are also indefinite because claim 21 recites the phrase "between four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, and twenty amino acids". The phrase renders the claim indefinite because it is unclear whether the phrase is meant to recite a range or ranges of values. Moreover, it is unclear what the values of the limits of the range or ranges are and whether the range are intended to include values of less than whole integers. For example, it is clear that a linker cannot consist of 4.5 amino acids; however, the claim appears to include such a value, because the claim recites "wherein the linker is between 4 and 5 [...] amino acids". Therefore, one of ordinary skill in the art is not reasonably apprised of the metes and bounds of the invention. Amending the claim to recite, for example, the phrase "wherein the linker consists of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids" can obviate this rejection.

Claim 22 is also indefinite because the claim recites the phrase "wherein the linker is between five to ten". The use of the phrase renders the claim indefinite because it is unclear whether the limitation requires the linker to consist of a number of amino acids between 5 and 10 or a number of amino acids ranging from 6 to 10. In other words, as the claim is written, it is unclear whether the range of linker lengths is inclusive of 10 amino acids. Therefore, one of ordinary skill in the art is not reasonably apprised of the metes and bounds of the invention. Amending claim 22 to recite, for example, the phrase "wherein the linker is between five and ten" or the phrase "wherein the linker is at least 6 and up to 10" can obviate this rejection.

Claim 23-37 and 65 are also vague and indefinite because claim 23 recites the phrase "a fragment thereof". The use of the phrase renders the claim vague and

indefinite because it cannot be ascertained whether the claim requires the fragment to specifically bind the antigen to which the antibody specifically binds. Therefore, one of ordinary skill in the art is not reasonably apprised of the metes and bounds of the invention. Amending the claim to recite, for example, the phrase "the antigen-binding fragment thereof" can obviate this rejection.

Claim 24 is also vague and indefinite because the claim recites the phrase "capable of forming a tetrameric complex". The use of the phrase renders the claims vague and indefinite because it is unclear whether the fusion protein is required to form a tetrameric complex or is merely required to be capable of forming a tetrameric complex. It is also unclear how a tetrameric complex is required to form (e.g., covalently, non-covalently, etc.). It is also unclear what under what conditions the fusion protein is required to be capable of forming a tetrameric complex and whether the interaction between the fusion protein and the second, third, and fourth fusion proteins is specific or non-specific. Therefore, one of ordinary skill in the art is not reasonably apprised of the metes and bounds of the invention.

Claims 24 is also indefinite because the claim recites the phrase "a first and a second polypeptide joined end to end". The use of the phrase renders the claim indefinite because it is unclear whether the terms first polypeptide and second polypeptide are meant to indicate the amino-terminal polypeptide and the carboxyl-terminal peptide, respectively, or are merely meant to distinguish one from the other. The fact that the first and second polypeptides are joined end to end is not limiting with regard to the orientation of the polypeptides relative to one another within the fusion protein. Accordingly, one of ordinary skill in the art is not reasonably apprised of the metes and bounds of the invention. Amending claim 18 to recite, for example, the phrase "a first amino-terminal and a second carboxyl-terminal polypeptide joined end to end" can obviate this rejection.

Claims 26-37 are also indefinite because claim 26 recites the phrase "scFv" in parentheses. The use of the phrase in parentheses renders the claim indefinite because it is unclear whether the phrase is intended to be a limitation or is merely parenthetical in nature. If the claim is intended to be a limitation, claim 26 should be

amended to delete the parentheses and the phrase should be offset from the preceding word by a comma. If the phrase in intended to be parenthetical, the claim should be amended to delete the phrase.

Claims 33-37 are also vague and indefinite because the claim recites the phrase "is specific for a cell surface protein or a cell-associated stromal or matrix protein". The use of the phrase renders the claim vague and indefinite because it is not clear whether the antibody is required to specifically bind a cell surface protein or a cell-associated stromal or matrix protein. Therefore, one of ordinary skill in the art is not reasonably apprised of the metes and bounds of the invention. Amending claim 33 to recite, for example, the phrase "wherein the antibody specifically binds a cell surface protein or a cell-associated stromal or matrix protein" can obviate this rejection. However, it is also unclear whether the fusion protein of claim 33 is required to bind specifically to the antigen to which the antibody "is specific".

Claim 34 is also indefinite because of the use of the trademark "Primatized™". Where a trademark or trade name is used in a claim, MPEP 7.35.01 reads, "the claim scope is uncertain since the trademark or trade name cannot be used properly to identify any particular material product. On the other hand, a trademark or trade name is used to identify a source of goods, and not the goods themselves. Thus, a trademark or trade name does not identify or describe the goods associated with the trademark or trade name." See *Ex parte Simpson*, 218 USPQ 1020 (PTO Board of Patent Appeals and Interferences, 1982). Amending the claim to delete the trademark name "Primatized™" can obviate this rejection; however, Applicant is cautioned against the introduction of new matter into the claims and/or specification by amendment.

Claim Rejections - 35 USC § 103

15. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art

> are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

16. Claims 18-39 and 65 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dubel, et al (*Journal of Immunological Methods* **178**: 201-209, 1995; Form PTO-1449, Paper No. 6, page 3), as evidenced by Kipriyanov, et al (*Human Antibodies and Hybridomas* **6**: 93-101, 1995; Form PTO-1449, Paper No. 6, page 4), in view of Desplancq, et al (*Protein Engineering* **7**: 1027-1033, 1994; Form PTO-1449, Paper No. 6, page 3), Anderson, et al (*Clinical Immunology and Immunopathology* **84**: 73-84, 1997), McLaughlin, et al (*Oncology* **12**: 1763-1769, 1998), the Internet edition of the Bioprobe BV Catalog of Mouse Hybridomas (Bandung, Indonesia), Gallizia, et al (*Protein Expression and Purification* **14**: 192-196, 1998; Form PTO-1449, Paper No. 6, page 3), and Pahler, et al (*Journal of Biological Chemistry* **262**: 13933-13937, 1987), Aragarana, et al (*Nucleic Acids Research* **14**: 1871-1882, 1986; Form PTO-1449, Paper No. 6, page 1), Ohno, et al (*DNA and Cell Biology* **15**: 401-406, 1996; Form PTO-1449, Paper No. 6, page 5), and Goshorn, et al (*Cancer Research* **53**: 2123-2127, 1993; Form PTO-1449, Paper No. 6, page 3).

Claims 18-22 are drawn to a fusion protein comprising at least a first and a second polypeptide joined end to end, wherein the first polypeptide comprises at least 129 amino acids of streptavidin (claim 18) wherein said first and second polypeptides are separated by a linker of at least 2 or 4 amino acids (claims 19 and 20, respectively) wherein said linker consists of between 4 and 20 amino acids (claims 21) or between 5 and 10 amino acids (claim 22). Claims 23-32 are drawn to the fusion protein of claim 18 wherein said second polypeptide is an antibody or fragment thereof (claim 23) wherein said fusion protein is capable of forming a tetrameric complex with a second, third, and fourth fusion protein (claim 24) or wherein said antibody is B9E9 (claim 25) or wherein said antibody is a single-chain Fv antibody fragment (claim 26) wherein the single-chain Fv fragment is derived from antibody B9E9 (claim 27) or wherein a linker connects the variable light and variable heavy chains of the single-chain antibody (claim 28) wherein the linker comprises at least 10, 15, or 20 amino acids (claims 29, 30, and 31,

respectively) wherein said 20 amino acid linker comprises at least four gly_4ser linkers (claim 32). Claims 33-37 are drawn to the fusion protein of claim 23 wherein the antibody is specific for a cell surface protein or a cell-associated stromal or matrix protein (claim 33) wherein the antibody is a Primatized™ antibody (claim 34), or wherein the antibody is a murine antibody (claim 35) or wherein the cell surface protein is CD20 (claims 36 and 37). Claims 38 and 39 are drawn to the fusion protein of claim 18 wherein the first polypeptide comprises at least amino acids 14 to 150 of streptavidin.

Dubel, et al teaches a first fusion protein capable of forming a tetrameric complex with a second, third, and forth fusion protein, wherein the fusion proteins comprise a first polypeptide comprising a portion of streptavidin and a second polypeptide comprising a fragment of an antibody that specifically binds a cell-associated stromal or matrix protein. Dubel, et al disclose that the fusion protein consists of single-chain antibody 215 joined end to end with core-streptavidin by a spacer (i.e., linker) of 5 amino acids (page 203, Figure 1). The variable light and variable heavy chains of the single-chain antibody are connected by a linker of 15 amino acids, as evidenced by Kipriyanov, et al (page 95, Figure 1). The single-chain antibody is derived from a mouse (i.e., murine) antibody, which specifically binds an epitope of RNA polymerase II, a cell-associated stromal or matrix protein (page 205, column 1). Dubel, et al teach that the fusion protein can form tetrameric complexes and binds antigen (abstract). Furthermore, Dubel, et al teach that "the variable region (Fv) portion of an antibody is comprised of its V_H and V_L domains and is the smallest antibody fragment containing a complete antigen binding site" (page 201, column 2). Dubel, et al teach that single-chain Fv antibodies (scFv) "represent potentially very useful molecules for the targeted delivery of drugs, toxins, or radionuclides to a tumour site" (page 201, column 2). Dubel, et al disclose that "various heterologous protein moieties can also be genetically fused to scFv antibodies to generate bifunctional fusion proteins" (page 202, column 1). Additonally, Dubel, et al teach that streptavidin "exhibits one of the strongest noncovalent binding affinities known for a biomolecule", namely biotin (page 208, column 1). Dubel, et al teach that the fusion protein comprising a single-chain antibody and streptavidin might be "usefully employed for the in vitro purging of autologous bone marrow transplants to

eliminate B lymphocytes in the treatment of leukemias and malignant lymphomas" (page 208, column 1).

However, Dubel, et al do not explicitly teach that the first polypeptide of the fusion protein can comprise at least 129 amino acid residues of streptavidin or at least amino acids 14 to 150 of streptavidin. Also, Dubel, et al do not explicitly teach that the fusion protein can comprise a linker connecting the first and second polypeptides that consists of between 5 and 10 amino acids. Dubel, et al do not explicitly teach that the antibody from which the fusion protein is derived can be Primatized™. Furthermore, Dubel, et al do not explicitly teach that the antibody from which the fusion protein is derived can bind specifically to CD20 or that the antibody can be produced by the hybridoma cell line B9E9, which produces the monoclonal antibody B9E9 that specifically binds CD20. Finally, Dubel, et al do not explicitly disclose that the linker connecting the variable light and variable heavy chains of the single-chain antibody can comprise at least 20 amino acids, wherein said linker comprises four gly_4ser linkers.

Kipriyanov, et al teach that the single-chain antibody-streptavidin fusion protein of Dubel, et al was developed "to increase the avidity of single-chain antibodies (scFv) for their antigen" (abstract). Kipriyanov, et al teach that the fact that the single-chain antibody-streptavidin fusion protein can form tetramers and therefore overcomes one limitation of scFv, which due to their monovalency have decreased avidity relevant to polyvalent antibodies (page 93, column 1). Kipriyanov, et al conclude, "the affinity of the scFv-antibody complex was substantially increased by avidity effects due to the tetrameric structure" (abstract). Also, Kipriyanov, et al disclose that "deletions of several amino acids from the N-/C-terminus resulting in a core-streptavidin molecule did not influence the biotin binding" (page 94, column 1).

Ohno, et al teach that tissue-specific delivery of a variety of molecules is a valuable technique for medical research (abstract). Ohno, et al disclose that "the cell-targeting moiety can be either antibodies or protein ligands (growth factors) that recognize the corresponding antigens or receptor (page 401, column 1). Ohno, et al demonstrate that a streptavidin-ligand fusion protein, ST-TGF-α, efficiently targets

biotinylated protein to cells that express the ligand's receptor (page 404, Figure 3). Ohno, et al teach that streptavidin-ligand and streptavidin-antibody fusion proteins have a number or advantages over immunotoxins and recombinant toxins for treatment of disease, namely cancer (pages 404-405). "Because biotin can be easily incorporated into a wide range of macromolecules without interfering with biological activities (Wilchek and Bayer, 1990) steptavidin containing-proteins such as ST-TGF-α have wider applicability as bridges to deliver specific molecules such as toxins" (page 405, column 1). Then, Ohno, et al teach that "other chimeric molecules in which the TGF-a moiety has been replaced by an alternate targeting element may have equally broad applicability to targeting a variety of cell types with equal affinities" (page 405, column 2).

Gallizia, et al teaches a method for producing a fusion protein comprising a first and a second polypeptide joined end to end, wherein the one of the two polypeptides comprising said fusion protein comprises at least 129 residues of streptavidin. Specifically, Gallizia, et al teach that one of the two polypeptides comprising the fusion protein consists of amino acids 15 to 159 of streptavidin (page 193, column 2) while the other polypeptide consists of amino acid sequence that differs by at least one residue from the other polypeptide. Gallizia, et al teach that the fusion protein comprising a portion of streptavidin retains the functional capacity to bind iminobiotin (page 196, column 1).

Pahler, et al teach that streptavidin is "known to be susceptible to proteolysis near the N terminus" (page 13934, column 1). Pahler, et al teach that streptavidin is cleaved at or near residue 14, close to the amino-terminus, within its amino acid sequence (page 13394, Figure 1). Pahler, et al also teach that the carboxyl-terminal of streptavidin is susceptible to protease activity (page 13934, column 1). Also, Pahler, et al teach that "core streptavidin", which has been proteolytically truncated at both ends, retains its ability to bind biotin (abstract).

Desplancq, et al teach that "thirteen scFv variants with linkers comprising up to six repeats of the motif Gly-Gly-Gly-Gly-Ser were studied" (abstract). Desplancq, et al disclose that "the V_L-linker-V_H variant with a 30 amino acid linker showed slightly poorer

binding activity than the monovalent F(ab') standard" (page 1030, column 2). Desplancq, et al teach that "precipitation problems can be overcome by utilizing loner linkers" (page 1033, column 1). Desplancq, et al also teach that single-chain Fv antibodies "are of interest for clinical applications because their pharmacokinetics and biodistribution may be superior to those of whole antibodies in some clinical applications" (page 1027, column 1).

Goshorn, et al teach the production and use of a fusion protein comprising a first polypeptide and a second polypeptide, wherein the first and second polypeptides are joined end to end, but separated by a linker of 6 amino acids, wherein one of the polypeptides is a single-chain antibody (page 2124, Figure 1). Goshorn, et al teach that the fusion protein is able to bind specifically to tumor cells displaying the antigen to which the antibody from which the fusion protein is derived binds (page 2126, Figure 3). Goshorn, et al also teach that the second polypeptide of which the fusion protein is comprised retains its capacity to bind substrate (page 2126, Figure 4). Furthermore, Goshorn, et al teach that "a promising new approach for tumor therapy is to use the antigen-binding capability of an antibody to deliver enzymatic activities to tumor tissues, which are then exploited to convert relatively nontoxic prodrugs into more active chemotherapeutic agents" (page 2123, column 1).

Anderson, et al teach the production and use of a Primatized™ monoclonal antibody (abstract). Anderson, et al teach that the use of Primatized™ monoclonal antibodies circumvents the problems associated with administration of xenogeneic monoclonal antibodies to treat human diseases, such a anti-mouse human immune response, which limits the applicability and efficacy of pharmaceutical compositions comprising mouse monoclonal antibodies (page 74, column 1).

McLaughlin, et al teach the clinical status and optimal use of Rituximab™, a recombinant humanized monoclonal antibody that specifically binds CD20 (abstract). Mclaughlin, et al disclose that "as the first MoAb [monoclonal antibody] to gain FDA approval for the treatment of a malignancy, rituximab signals the beginning of a promising new era in cancer therapy" (abstract).

The Bioprobe BV catalog demonstrates that the hybridoma that produces the anti-CD20 monoclonal antibody B9E9 is commercially available (page 5 of the Internet published catalog).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the teachings of Dubel, et al to produce and use a fusion protein comprising a first polypeptide and a second polypeptide, wherein the first polypeptide comprises at least 129 amino acid residues of streptavidin or at least amino acids 14 to 150 of streptavidin, because Arragarana, et al teach that the first 24 amino-terminal amino acids compose a signal sequence that is cleaved and because Pahler, et al teach that about the next 13 amino acids of streptavidin are susceptible to protease cleavage. Therefore, if one of ordinary skill in the art were to be motivated to produce a fusion protein comprising an amino-terminal first polypeptide, wherein the first polypeptide is a fragment of streptavidin, it would have been *prima facie* obvious, in light of the teachings of Pahler, et al, to truncate the amino-terminus of streptavidin, in order to decrease the susceptibility of the free amino-terminus to protease activity while retaining as much of the structure of streptavidin as possible to insure the maintenance of its biotin-binding capacity. Additionally, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the teachings of Dubel, et al to produce and use a fusion protein comprising a first polypeptide and a second polypeptide, wherein the first and second polypeptides are separated by a linker that is between five and ten amino acids, because Goshorn, et al teach that a fusion protein comprising a first and a second polypeptide can be produced, wherein the first and second polypeptides are separated by a linker of 6 amino acids, which retains the ability to bind specifically to the antigen to which the antibody from the which the fusion protein is derived binds and which retains the ability to bind specifically to the substrate to which the second polypeptide from which the fusion protein is derived binds. It would also have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to further modify the teachings of Dubel, et al to produce and use a fusion protein comprising a first polypeptide and a second polypeptide, wherein the first polypeptide is an antigen-

binding fragment of the monoclonal antibody B9E9, because monoclonal antibody B9E9 specifically binds CD20 and is commercially available and easily obtained. One of ordinary skill in the art would appreciate the fact that, based upon the teachings of Dubel, et al, the fusion of an antigen-binding fragment of the monoclonal antibody B9E9 and a second polypeptide could be used to selectively and specifically target the second polypeptide, which is fused to the first polypeptide, to a CD20+ lymphoma tumor cell, because McLaughlin, et al teach that anti-CD20 antibody-directed therapy can be used effectively to treat patients diagnosed with lymphoma. In light of the teachings of Dubel, et al Desplancq, et al, and Goshorn, et al, for example, the selectivity and binding specificity of monoclonal antibodies and antigen-binding fragments thereof was well known in the art at the time the invention was made. Because the second polypeptide of Dubel, et al is a biotin-binding fragment of streptavidin, one of ordinary skill in the art would appreciate the fact that the fusion protein could be used to selectively target biotinylated-chemotherapeutic agents to CD20+ cells bound specifically by the antibody portion of the fusion protein, because according to Dubel, et al, streptavidin has a very high affinity for biotin. Because Dubel, et al teach that the fusion protein forms tetramers, one of ordinary skill in the art would appreciate the fact that four biotinylated molecules could potentially be targeted to the CD20+ lymphoma tumor cells. Furthermore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to produce a fusion protein, according to the methodology of Dubel, et al, comprising a single-chain Fv derived from the monoclonal antibody B9E9, because Dubel, et al teaches that scFv are the smallest fragments of an antibody to retain specific antigen-binding capacity and therefore, as Desplanqc, et al teach, the pharmacokinetics and biodistribution of scFv may be superior to those of whole antibodies. In light of the teachings of Anderson, et al, it also would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to further modify the teachings of Dubel, et al to produce a fusion protein comprising a single-chain Fv derived from a recombinant Primatized™ or humanized antibody derived from the mouse monoclonal antibody B9E9, because Anderson, et al teaches that

Primatized™ antibodies overcome limitations caused by the immunogenicity of xenogeneic antibodies formulated as pharmaceutical compositions for use in humans. Finally, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to further modify the fusion protein of Dubel, et al to separate the variable and heavy chains of the scFv antibody component of the fusion protein, because Desplanqc, et al teach that a scFv antibody consisting of variable heavy and light chains separated by a linker of at least 20 amino acids, wherein the linker consists of at least four gly_4-ser motifs, maintains antigen-binding specificity and because Desplanqc, et al teach that the use of a longer linker may overcome problems that occur during production of the single-chain antibodies.

One of ordinary skill in the art would have been motivated to modify the fusion protein of Dubel, et al, according to the teachings of McLaughlin, et al, Anderson, et al, and Desplanqc, et al, to produce a recombinant Primatized™ single-chain anti-CD20 B9E9 antibody-streptavidin fusion protein, because there is a long-felt need for a more effective therapy for lymphoma, because the hybridoma producing the recombinant antibody B9E9 is commercially available and would be required to produce the recombinant antibody-streptavidin fusion protein, and also because Dubel, et al teach that single-chain antibody-streptavidin fusion proteins can be used to treat lymphoma, McLaughlin, et al teach that anti-CD20 antibody therapy can be used effectively treat lymphoma, Desplancq, et al teach the advantages of using scFv antibodies, and Anderson, et al teach the advantages of using Primatized™ antibodies in treating humans. One of ordinary skill in the art would have been motivated at the time the invention was made to construct an expression cassette encoding a fusion protein comprising at least 129 amino acids of streptavidin, including at least amino acids 14-150, because the first 24 amino-terminal amino acids of the translation product compose a signal sequence that is cleaved, according to the teachings of Aragarana, et al, and also because the further truncation of the amino-terminus or carboxyl-terminus of the streptavidin portion of the fusion protein would limit the fusion protein's susceptibility to protease activity without effecting its biotin-binding capacity, according

to the teachings of Pahler, et al. Also, one of ordinary skill in the art would have been motivated at the time the invention was made to separate the first and second polypeptides of the fusion protein, namely streptavidin and the single-chain antibody, because the insertion of a linker would facilitate insertion of a multiple cloning site into vector encoding the fusion protein so that the segment of the vector encoding the single-chain antibody can be easily replaced by another segment encoding a different single-chain antibody. Finally, one of ordinary skill in the art would have been motivated at the time the invention was made to separate the variable heavy and light chains of the single-chain Fv antibody by a linker consisting of four gly-gly-gly-gly-ser linkers, because Desplancq, et al teach that problems with precipitation that are encountered during the manufacture of recombinant antibodies might be overcome by the use of a longer linker separating the variable heavy and light chains of the scFv antibody.

Conclusion

17. No claims are allowed.

18. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

Axworthy, et al (*Journal of Nuclear Medicine* **33**: 880, 1992) teach a diagnostic method for imaging tumors in a subject comprising administering biotinylated pan-carcinoma NR-LU-10 monoclonal antibody.

Hnatowich, et al (*Journal of Nuclear Medicine* **28**: 1294-1302, 1987) teach a diagnostic method for imaging tumors in a subject comprising administering a biotin-conjugated antibody before administering radiolabeled avidin or streptavidin. Hnatowich, et al also teach a diagnostic method for imaging tumors in a subject comprising administering an avidin-conjugated antibody before administering radiolabeled biotin.

Axworthy, et al (*Proceedings of the National Academy of Science USA* **97**: 1802-1807, 2000) teach a method for using a covalent conjugate of streptavidin and a monoclonal antibody to treat mice bearing tumors.

19. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stephen L. Rawlings, Ph.D. whose telephone number is (703) 305-3008. The examiner can normally be reached on Monday-Thursday, alternate Fridays, 8:00AM-5:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony C. Caputa, Ph.D. can be reached at (703) 308-3995. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 for regular communications and (703) 308-4242 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Stephen L. Rawlings, Ph.D.
Examiner
Art Unit 1642

DONNA WORTMAN
PRIMARY EXAMINER

slr
June 15, 2001